## NHS-activated Sepharose 4 Fast Flow Prepacked media

**NHS-activated Sepharose™ 4 Fast Flow, appropriate for process-scale applications, is for coupling amino-containing proteins and peptides with a chemically-stable linkage**

- High level of activation can give a high degree of substitution of the selected ligand
- Ligand coupling results in chemically-stable ligand
- Sepharose Fast Flow matrix allows high flow rates at moderate pressures

Technical Data

| | |
|---|---|
| Composition | highly cross-linked 4% agarose |
| Particle size | 45-165 µm |
| For coupling to | $-NH_2$ |
| Active groups | NHS ester of carboxylic acid groups on 10-atom spacer |
| Amount of active groups | ~ 18 µmol NHS/ml drained medium |
| pH stability (operational) | 3-13, ligand dependent |
| CIP stability (short term) | 3-13, ligand dependent |
| Pressure/flow spec | base matrix 150-250 cm/h, 1 bar (100 kPa), XK 50/60 column, bed height 25 cm |

Ordering Information

| Product | Pack Size | Code no |
|---|---|---|
| NHS-activated Sepharose 4 Fast Flow | 25 ml | 17-0906-01 |
| -//- | 500 ml | 17-0906-02 |
| -//- | 5 l | 17-0906-04 |
| **Prepacked columns** | | |
| HiTrap NHS-activated HP columns 1x5 ml | | 17-0717-01 |
| HiTrap NHS-activated HP columns 5x1 ml | | 17-0716-01 |

data file
Affinity chromatography

# HiTrap NHS-activated 1 ml and 5 ml

HiTrap™ is a range of pre-packed, ready-to-use columns for preparative affinity chromatography. Fast, simple and easy separations are provided by the combination of a specially designed column and a high performance affinity medium. HiTrap NHS-activated is the pre-activated member of this column family. The coupling method supplied is easy to perform, even if you have never coupled a gel before. From starting the method to a ready-to-use affinity gel normally takes less than an hour. HiTrap NHS activated is available in 1 ml and 5 ml bed volumes.

- Fast and convenient to use
- Packed with NHS-activated Sepharose™ High Performance
- Simple operation with a syringe, a pump or a chromatographic system like ÄKTA™ or FPLC™
- Affordable

Coupled HiTrap NHS-activated columns can easily be operated using a syringe. Alternatively, a laboratory pump, alone or within a chromatography system, can be advantageous, especially when linear gradients are required.

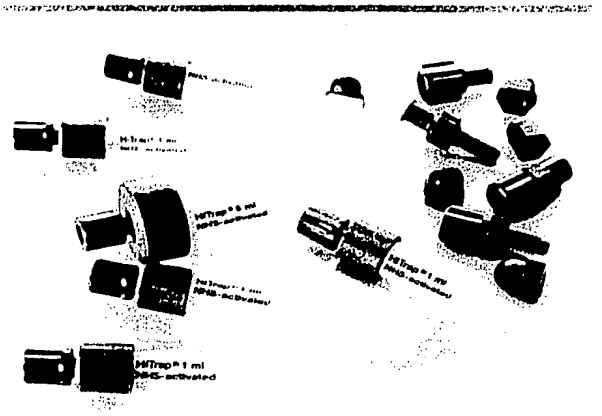


**Fig. 1.** Prepacked with NHS-activated Sepharose High Performance, HiTrap NHS-activated columns allow easy ligand coupling that opens up a wide range of uses.

## Column characteristics

The HiTrap column is made of medical grade polypropylene, a material that is biocompatible and does not interact with biomolecules. Top and bottom frits are manufactured from porous polyethylene. The column is delivered with a stopper on the inlet and a twist-off end on the outlet. Both ends have M6 connections (6 mm metric threads).

## Media characteristics

Sepharose High Performance is the base matrix for HiTrap NHS-activated. The carbohydrate nature of the agarose base provides a hydrophilic and chemically favourable environment for coupling, while the highly cross-linked structure of the 34 µm spherical beads ensures excellent chromatographic properties. Fast kinetics and high dynamic capacities are properties of all HiTrap affinity columns.

HiTrap NHS-activated comprises N-hydroxy-succinimide (NHS) ester attached by epichloro-hydrine to Sepharose High Performance via a 6-atom spacer arm. This esterification leads to the formation of activated esters, which react rapidly and efficiently with ligands containing amino groups to give a very stable amide linkage. The active esters are stable in the absence of water.

HiTrap NHS-activated is supplied in 100% isopropanol to preserve its activity prior to coupling. Table 1 lists the main characteristics of HiTrap NHS-activated.

(df) 18-1134-80 AB, 2000-05 • p1

Amersham Biosciences

| | |
|---|---|
| Column dimensions | 0.7×2.5 cm (1 ml),<br>1.6×2.5 cm (5 ml) |
| Ligand | NHS groups |
| Ligand concentration | 10 µmol/ml |
| Mean particle size | 34 µm |
| Bead structure | Highly cross-linked<br>spherical agarose |
| Max. back pressure | 0.3 MPa, 3 bar |
| Max. flow rate | 4 ml/min (1 ml),<br>20 ml/min (5 ml) |
| Recommended flow rate | 1 ml/min (1 ml),<br>5 ml/min (5 ml) |
| pH stability* | |
| Regular use | 3–12 |
| Cleaning | 3–12 |
| Temperature stability | |
| Regular use | +4 °C to room temp. |
| Storage | +4 to +8 °C |
| Storage buffer | 100% isopropanol |

* The ranges given are estimates based on our knowledge and experience. Please note the following:

i) pH stability regular use refers to the pH interval where the gel is stable over a long period of time without adverse effects on its subsequent chromatographic performance.

ii) pH stability cleaning refers to the pH interval for regeneration, cleaning-in-place and sanitization procedures.

iii) Both intervals refer to the coupled product provided that the ligand can withstand the pH.

**Table 1.** Main characteristics of HiTrap NHS-activated.

## Coupling procedure

Ligands containing primary amino groups are easy to attach to HiTrap NHS activated, allowing a wide range of uses that require the pinpoint specificity of a laboratory coupled affinity gel. Table 2 describes a basic coupling procedure.

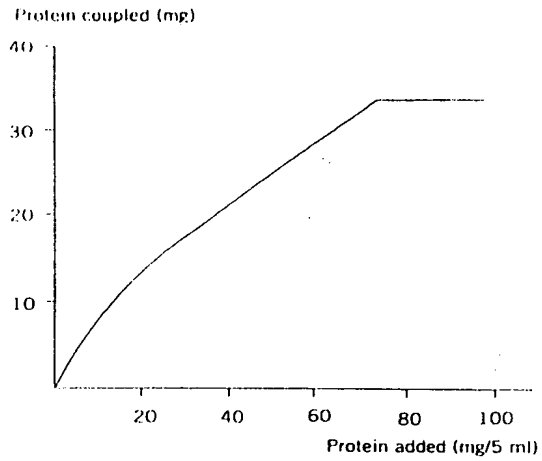



**Fig. 2.** Coupling 30 mg IgG to a HiTrap NHS-activated 1 ml column takes less than 15 minutes.

| Step | Notes |
|---|---|
| 1. Dissolve the desired ligand in the coupling buffer to the desired concentration. | The concentration depends on the ligand being used. As a general rule: 5–10 mg/ml of ligands containing primary amino groups; the optimal volume is one column volume. |
| 2. Remove the top-cap. | Apply a drop of 1 mM HCl to the top of the column to avoid air bubbles. |
| 3. Connect the HiTrap luer adaptor (or tubing from a system) to the column. Remove the twist-off end. Wash out the isopropanol gently. | Use 6 column volumes 1 mM HCl at approx. 1 ml/min or 1 drop/3 sec. |
| 4. Immediately inject one column volume of the ligand solution onto the column. | Do not allow the column to stand at this point or its coupling activity will be lost. |
| 5. Close the column and allow coupling to take place. | 15–30 minutes at +25 °C (or 4 hours at +4 °C). If large volumes of ligand solution are used, connect a second syringe to the outlet of the column and gently pump the solution back and forth for 10–15 minutes. A peristaltic pump, e. g. Pump P-1, can also be used. |
| 6. Deactivate any excess active groups. | Inject 6 column volumes of high pH buffer (0.5 M ethanolamine, 0.5 M NaCl, pH 8.3), followed by 6 column volumes of low pH buffer (0.1 M acetate, 0.5 M NaCl, pH 4). Repeat the washing with high pH buffer and allow the column to stand for 15–30 min. |
| 7. Wash out uncoupled ligands. | Complete the washing cycle with repeating low and high pH washes as above, followed by an appropriate storage buffer. |
| 8. The column is now ready for use; store at +4 to +8 °C. | |

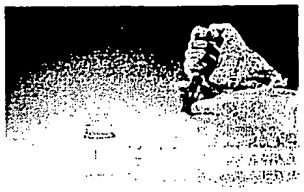
**Table 2.** A basic coupling procedure using a syringe for HiTrap NHS-activated.

Figure 2 shows that over 30 mg IgG can be coupled to a HiTrap NHS-activated column in less than 15 minutes. Further studies have shown that ligands can be coupled in the presence of detergents such as 0.1% SDS, Triton™ X-100, Tween™ 20 and sodium deoxycholate, and that the yield is independent of pH in the range 7–10.

## Operation

Like all HiTrap columns, HiTrap NHS-activated is quick and easy-to-use. Instructions and connectors are included with each pack of columns. In general, the separation can be easily achieved with a syringe (using the luer adaptor provided). Figure 3 illustrates this technique. Alternatively, the column can be operated using a laboratory pump via an M6 tubing fitting. Two or more columns can be connected in series by screwing the end of one into the top of the next.

a) b)



c)

**Fig. 3.** Using HiTrap NHS-activated with a syringe. a) Prepare buffers and sample. Remove the column's top cap and twist off the end. Wash and equilibrate. b) Load the sample and begin collecting fractions. c) Elute and continue collecting fractions.

## Applications

The following applications show work done on HiTrap NHS-activated columns coupled with different ligands using different methods. These methods, the single application of coupling solution by a syringe, recirculation with two syringes, and recirculation with a peristaltic pump, are all supplied with the pre-activated column. All methods gave excellent yields and good separations.

### Partial purification of an IgE-stimulating factor from a human T-cell line.

A factor stimulating the production of IgE (but probably not one of the known interleukins) was found in an IgE-producing myeloma. Initial purification of the factor was attempted on IgE coupled to HiTrap NHS-activated 1 ml. 1 ml (5 mg) IgE was coupled to HiTrap NHS-activated 1 ml with a syringe. After 1 hour, the column was washed. 70% of the IgE (3.5 mg) was coupled to the matrix. Figure 4 shows the subsequent separation conditions and result.

Increased production of IgE in cell line U-266 was only found when the cells were grown in the presence of desorbed material. $B2\mu$ levels, a reflection of total protein synthesis, showed that the increased IgE production was not due to an overall stimulus of the cell line.

### Removal of BSA from an HIV-2 virus lysate

Work to purify *gag* proteins from an HIV-2 virus lysate was hampered by the binding of BSA present in the virus culture to a RPC column. This BSA could not be removed completely from the column by regeneration and interfered with the HPLC purification of the viral proteins. Anti-BSA antibodies were therefore purified and coupled to HiTrap NHS-activated in an attempt to remove BSA from the lysate prior to running on the RPC column.

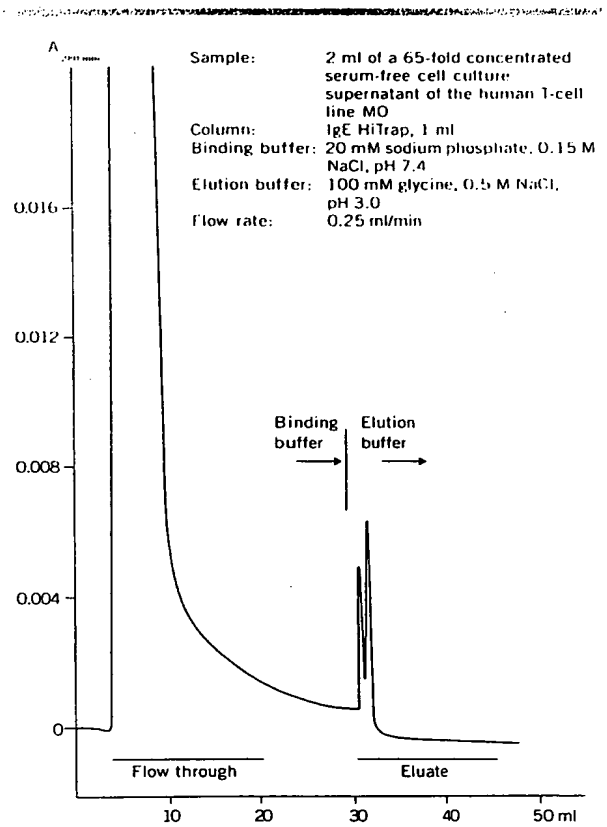



**Fig. 4.** Separation of an IgE-stimulating factor from a human T-cell line on a 1 ml IgE HiTrap column.

Coupling was carried out according to a method supplied with the column. Coupling solution was recirculated for 20 minutes at a flow rate of about 2 ml/min by manually pumping it back and forth through the column with two syringes. 95% (81 mg) of the anti-BSA was coupled by this method.

To test the efficiency of the BSA removal, 14.1 mg BSA were passed through the anti-BSA HiTrap column. 0.45 mg (3.2%) was detected in the flow through and 13.54 mg (96.0%) had bound. Total recovery of bound and non-bound BSA was 99.2%. As BSA comprised at the most ¼ of the total protein as judged by electrophoresis, more than 99% of the BSA applied to the column had probably bound.

When the HIV-2 virus lysate that passed through the anti-BSA HiTrap 1 ml column was now run on the RPC column, the separation was completely free from interference by BSA.

### Purification of anti-mouse Fc IgG from sheep anti-serum

Mouse IgG (10 mg, 3 mg/ml) was coupled to HiTrap NHS-activated at room temperature for 50 minutes by recirculation with a peristaltic pump. 9.5 mg (94.9%) was coupled.

The column was then used for the purification of anti-mouse Fc IgG from sheep antiserum with excellent results.

## Ordering Information

| Product | Pack | Code No. |
|---|---|---|
| HiTrap NHS-activated | 1 mlx5 | 17-0716-01 |
| HiTrap NHS-activated | 5 mlx1 | 17-0717-01 |
| NHS activated Sepharose 4 Fast Flow | 25 ml | 17-0906-01 |

***Accessories***

| Product | Pack | Code No. |
|---|---|---|
| Domed nut* | 4 | 18-2450-01 |
| Union luerlock | | |
| female/M6 female* | 2 | 18-1027-12 |
| female/M6 male* | 2 | 18-1027-62 |
| Tubing connector | | |
| flangeless/M6 female* | 2 | 18-1003-68 |
| flangeless/M6 male* | 2 | 18-1017-98 |
| To connect columns with M6 connections to ÄKTA design | | |
| union female/1/16" male | 5 | 18-3858-01 |

* Included in HiTrap package

*to order:*

**Asia Pacific** Tel: +852 2811 8693 Fax: +852 2811 5251 **Australasia** Tel: +61 2 9894 5152 Fax: +61 2 9899 7511 **Austria** Tel: 01 576 0616 20 Fax: 01 576 0616 27 **Belgium** Tel: 0800 73 888 Fax: 03 272 1637 **Canada** Tel: 1 800 463 5800 Fax: 1 800 567 1008 **Central, East, South East Europe** Tel: +43 1 982 3826 Fax: +43 1 985 8327 **Denmark** Tel: 45 16 2400 Fax: 45 16 2424 **Finland** Tel: 09 512 3940 Fax: 09 512 1710 **France** Tel: 0169 35 67 00 Fax: 0169 41 9677 **Germany** Tel: 0761 4903 401 Fax: 0761 4903 405 **Italy** Tel: 02 27322 1 Fax: 02 27302 212 **Japan** Tel: 81 3 5331 9336 Fax: 81 3 5331 9370 **Latin America** Tel: +55 11 3667 5700 Fax: +55 11 3667 87 99 **Middle East and Africa** Tel: +30 (1) 96 00 687 Fax: +30 (1) 96 00 693 **Netherlands** Tel: 0165 580 410 Fax: 0165 580 401 **Norway** Tel: 2318 5800 Fax: 2318 6800 **Portugal** Tel: 01 417 7035 Fax: 01 417 3184 **Russian Federation** Tel: +7 (095) 232 0250,956 1137 Fax: +7 (095) 230 6377 **South East Asia** Tel: 60 3 724 2080 Fax: 60 3 724 2090 **Spain** Tel: 93 594 49 50 Fax: 93 594 49 55 **Sweden** Tel: 018 612 19 00 Fax: 018 612 19 10 **Switzerland** Tel: 01 802 81 50 Fax: 01 802 81 51 **UK** Tel: 0800 616 928 Fax: 0800 616 927 **USA** Tel: +1 800 526 3593 Fax: +1 877 295 8102



Produced by Wiltstörm, Sweden 1000891, May, 2000
Printed matter, Licence 341 051



Amersham Biosciences